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URUSHIOLS OF POISONOUS ANACARDIACEAE

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Key Word Index—*Toxicodendron*; sp.; *Metopium toxiferum*; Anacardiaceae; poison ivy; poison oak; poison sumac; poison wood; GC–MS; *bis*-TMSi-alk(en)ylcatechols; urushiol.

Abstract—GC–MS has been used to analyze and characterize the mixture of *bis*-trimethylsilyl derivatives of 3-*n*-alk(en)ylcatechols (urushiol) obtained from certain poisonous members of the Anacardiaceae. Analyses revealed a variation in composition of urushiol obtained from the same species. Furthermore, urushiols from poison ivy and poison wood, while consisting largely of *n*-C_{15}-substituted catechols, also contain varying amounts of the homologous *n*-C_{17}-substituted catechols. Similarly, the 3-alk(en)ylcatechol mixtures from poison oak, while containing mainly *n*-C_{17}-species, also contain varying amounts of the *n*-C_{15} species. The analysis of a single poison sumac sample revealed that it contained predominantly 3-*n*-pentadec(en)ylcatechols.

INTRODUCTION

Certain members of the Anacardiaceae are considered "poisonous", in that contact with the skin sensitizes most individuals, producing delayed contact dermatitis upon subsequent encounter. Five members of the genus *Toxicodendron* produce the majority of these cases in the United States. They are poison ivy *T. radicans* and *T. rydbergii*), Eastern poison oak (*T. toxicarium*), Western poison oak (*T. diversilobum*), and poison sumac (*T. vernix*). Two other members of this family are the Japanese lac tree (*T. vernicifulum*), of commercial importance in the Orient, and poison wood (*Metopium toxiferum*), a bush which is native to South Florida and the Northern West Indies. The systematics and ecology of these genera have been extensively studied by Gillis [1].

Khittel [2], in 1858 suggested that the poison oak allergen was a mixture of volatile alkaloids but in 1915 Majima [3, 4] showed that the toxic principle obtained from the Japanese lac tree, which was termed "urushiol", was a mixture of four closely related 3-*n*-alk(en)ylcatechols, all of which contained a fifteen carbon atom side chain. The first unequivocal demonstration of a chemical relationship between the allergens obtained from different *Toxicodendron* species was by Hill *et al.* [5], who found that upon catalytic hydrogenation, Japanese lac urushiol and the toxic poison ivy principle both gave rise to 3-*n*-pentadecylcatechol. Because of this apparent relationship between lac and poison ivy urushiols, and a suggestion by McNair [6, 7] that the poison oak and poison ivy urushiols were the same, it became widely assumed that the vesicating principles of many *Toxicodendron* species were identical [8–10]. Later work [11, 12] based on chromatographic and degradation studies, showed that poison ivy urushiol was composed of four

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alk(en)ylcatechols, namely 3-*n*-pentadecylcatechol (2%), 3-*n*-8-pentadecylcatechol (10%), 3-*n*-(8,11-pentadecadienyl)catechol (64%), and 3-*n*-(8,11,14-pentadecatrienyl)catechol (23%). Sunthankar and Dawson [13] have also reported that while lac urushiol is also a mixture of 3-*n*-pentadec(en)ylcatechols, the composition of the mixture [with respect to proportions of each alk(en)ylcatechol] and the structure of the trienyl-component (with respect to double bond position and possibly stereochemistry) is different from that of poison ivy urushiol. More recently, it has been suggested [14] that poison oak urushiol differs from poison ivy urushiol, in that it is composed of a mixture of heptadec(en)ylcatechols.

Extracts of poison ivy and poison oak are widely used in the diagnosis and prophylactic treatment of sensitivity. Despite the wide use of these preparations, they have been insufficiently characterized by most analytical methods. This paper describes the application of GC–MS to the comparative analysis and characterization of the TMSi derivatives of urushiol samples obtained from different *Toxicodendron* and *Metopium* species, as well as different urushiol samples obtained from the same *Toxicodendron* species.

RESULTS

The data of Table 1 were obtained from elution profiles from eleven urushiol samples (seven from poison ivy, two from poison oak, and one each from poison sumac and poison wood). The results show that poison ivy urushiol is not only composed of pentadec(en)ylcatechols, but also contains a significant proportion of heptadec(en)ylcatechols. Similarly, poison oak urushiol is composed of both heptadec(en)yl and pentadec(en)ylcatechols.

According to Dawson [11, 14] poison ivy urushiol generally exhibits average double bond values (d.b.v.) in the range of 1·6–2·0. These values were obtained from quantitative hydrogenation data and reflect the average number of ethylenic double bonds per alk(en)ylcatechol molecule. Using the data of Table 1, average d.b.v. can be calculated using weighted averages. With the exception of sample MI-1, these calculated values fall within the range proposed. Corbett [15] has reported an unusually high d.b.v. of 2·9 for this sample, which is in reasonable agreement with our calculated value of 2·5.

It has been reported [12] that the content of 3-*n*-pentadecylcatechol in poison ivy urushiol is usually about 2–4% but four of the seven poison ivy samples studied (Table 1) did not contain detectable quantities of this compound. One sample (CI-1) contained only 0·2% of 3-*n*-heptadecylcatechol. Both of the poison oak urushiol samples examined contained significant amounts of pentadec(en)ylcatechols but the heptadecatrienylcate-

Table 1. Composition of urushiols from *Toxidendra* sp. and *Metopium toxiferum*

Urushiol sample	*Bis*-TMSi-C_{15}-Catechols				*Bis*-TMSi-C_{17}-Catechols				
	Molecular weight								
	464	462	460	458	492	490	488	486	484
	Side chain								
	Saturated	Monoene	Diene	Triene	Saturated	Monoene	Diene	Triene	Tetraene
Poison ivy									
CI-1	0	25·8	67·8	3·3	0·2	0·7	1·8	0·4	0
CI-2	0	41·6	50·3	3·4	0	0·8	3·1*	0·8	0
CI-3	6·0	13·5	63·0	16·5	0	0·3	0·7	0	0
MI-1	0	15·4	17·9	62·5	0	0	0	4·2	0
FI-1	7·6	25·2	38·8	4·3	0	0·8	22·0*	1·3	0
FI-2	0	3·1	83·1	9·4	0	0·4	2·6*	1·4	0
FI-3	6·0	11·6	60·0	12·5	0	2·1	6·7*	1·1	0
Poison oak									
CO-1	22·3	8·4	2·7	0	2·1	10·7	18·7	35·1	0
MO-2	3·4	1·4	0·2	0·6	0	5·7	24·9	62·5	1·3
Poison sumac									
CS-1	15·3	41·4	32·7	10·6*	0	0	0	0	0
Poison wood									
CW-1	3·9	48·6	41·4	0	0	1·8	3·3†	1·0	0

* Two isomeric compounds of different R_t were observed for this M^+.
† Three isomeric compounds of different R_t were observed for this M^+.

chols predominated; sample CO-1 contained 33% 3-*n*-pentadec(en)ylcatechols and sample MO-1 6%. Corbett has also found evidence for the existence of pentadec(en)ylcatechols in a sample of poison oak urushiol [15]. In contrast to poison ivy, 3-*n*-pentadecylcatechol predominated in the C_{15}-substituted catechols of poison oak. The average d.b.v of poison oak urushiol is reported to be about 2·5. Sample CO-1 exhibited a calculated d.b.v. of 1·7, due to its high proportion of 3-*n*-pentadecylcatechol. However, poison oak urushiol sample MO-1 exhibited a calculated d.b.v. of 2·5, which was in good agreement with the experimental value of 2·6 [15]. It is of interest that a heptadecyltetraenylcatechol component was detected in sample MO-2. The single poison sumac urushiol sample CS-1 was the first sample encountered which consisted predominantly of 3-*n*-pentadec(en)ylcatechols. However, even this sample, as well as sample CI-3 contained trace levels (<0·2%) of both 3-*n*-heptylcatechol (R_t equivalent to a $C_{17\cdot4}$ hydrocarbon) and 3-*n*-tridecylcatechol (R_t equivalent to a $C_{25\cdot2}$ hydrocarbon); these structures are based on both their GLC R_t and MS. The sample also appeared to contain two pentadecatrienylcatechol isomers. Poison wood urushiol was very similar in composition to most of the poison ivy samples. It also contained about 5% of heptadec(en)ylcatechols, of which the dienyl components predominated. This sample was the only one encountered in which there was an indication of three isomeric 3-*n*-pentadecadienylcatechols of different R_t and it also contained trace levels (<0·2% of 3-*n*-nonanylcatechol (R_t equivalent to a $C_{19\cdot4}$ hydrocarbon)).

All of the catechol TMSi ethers exhibited fragmentation patterns analogous to the 3-*n*-pentadecylcatechol TMSi ether model. Alk(en)yl-3-catechols as a class are identified by the presence of three promient MS peaks in addition to the prominent M^+. A *bis*-TMS-1,2,dihydroxybenzyl ion at *m/e* 267 is formed in all cases by loss of alk(en)yl side chains from the M^+ by benzylic cleavage. This ion is accompanied by an ion at *m/e* 268, presumably due to γ-hydrogen rearrangement. A metastable ion was detected for this rearrangement (e.g. *m/e* 155 in the case of 3-*n*-pentadecylcatechol). Loss of tetramethylsilane from the *m/e* 268 ion produces an ion at *m/e* 179 in a further rearrangement characteristic of catechols TMSi ethers as opposed to the 1,3-and 1,4-dihydroxybenzene derivatives [16].

DISCUSSION

Immunologic studies [17] have shown that humans exhibit a differential sensitivity to the four (sic) pentadec(en)ylcatechols of poison ivy urushiol. 3-*n*-Pentadecylcatechol reacted in only 35% of sensitive individuals, while the dienyl- and trienyl-components produced dermatitis in almost all individuals tested. The pentadecenylcatechol component was intermediate in its allergenicity in humans. Thus, in view of these findings it would be useful to have an analytical method to characterize and standardize commerical oleoresin preparations used in the diagnosis and prophylactic treatment of poison ivy and poison oak sensitivity. The usefulness of the trimethylsilation GC–MS method is evident from its ability to reveal aspects of urushiol composition which have escaped prior detection (presence of homologs, isomers, and variation in compostion) but it does not reveal the position or stereochemistry of double bonds in the side chains of these molecules.

The allergenic principles of poison sumac and poison wood have been identified as alk(en)ylcatechols. Although only a single urushiol sample from each plant was examined, the composition of these mixtures appear to be similar to that of poison ivy urushiol. The poison sumac urushiol sample was of interest in that it was composed almost entirely of 3-*n*-pentadec(en)ylcatechols with traces of both 3-*n*-heptyl- and 3-*n*-tridecylcatechol.

The origin of the variability of urushiol composition from poisonous Anacardiaceae is probably of an environmental nature. Biosynthetic studies on the urushiols of poisonous Anacardiaceae have yet to be reported. The results presented here are consistent with a biosynthetic origin in which the common fatty acids are combined with three acetate units that eventually provide the catechol ring, as originally suggested by Geissman [18].

EXPERIMENTAL

The urushiol samples. Samples designated C [(I) ivy, (O) oak, (S) sumac, and (W) wood] were prepared by Professor Charles Dawson and co-workers at Columbia University, New York City. The poison ivy plants (*T. radicans*) used for these preparations were collected in Pearl River, New York. Poison oak

(*T. diversilobum*) originated in Northern California. Poison wood was obtained from the Bahama Islands and was identified by Dr. H. Irwin of the New York Botanical Gardens. Poison sumac (*T. vernix*) was collected from a swamp in Northern New Jersey by Dr. S. S. Ristrich of the Boyce Thompson Institute (Yonkers, NY). Samples designated M were prepared by Dr. M. Corbett and co-workers at the University of Mississippi, University, Mss. The poison ivy plants (*T. radicans*) used for these preparations were obtained in the area of the University. Samples designated F were prepared in the authors' laboratory at the Bureau of Biologics from plants (*T. radicans*) obtained in Bethesda, Maryland.

Preparation of urushiol samples. All samples were prepared by the same general extraction procedure [14]. Minced plants (excluding roots) were covered with *ca* 8× their wt of 95% EtOH and stirred under N_2 at 4° for 3 days. The extract was then concentrated *in vacuo* to 10% of its original vol, and the aq concentrate cooled at 4° for 24 hr and then filtered. The filtrate was extracted several times with an equal vol of C_6H_6. The C_6H_6 extract was washed with H_2O and evaporated under vacuum (10^{-1}mm) to a dark syrup (oleoresin). Samples designated F were not further purified. They contained 5–10% alk(en)ylcatechols as determined by preliminary trimethylsilylation-GLC analysis. Samples obtained from Prof. Dawson (C) were distilled under high vacuum *bp* 160–170° at 10^{-4}mm). Samples obtained from Dr. Corbett (M) were chromatographed on silicic acid (elution with C_6H_6–Et_2O 9:1).

Derivatization. One to five mg samples were dissolved in 0·1 ml of *bis*-(trimethylsilyl)trifluroacetamide (BSTFA) and maintained at 50° for 2 hr and then directly subjected to GC–MS analysis.

Analytical methods. GLC was performed with a 2 m × 2·5 mm helical glass column packed with 1% OV-17 and temp. programmed at 6–8°/min from ambient to 300°. R_t's were measured relative to a standard hydrocarbon mixture and are reported as hydrocarbon equivalents. MS were measured at 70 eV using an ionizing current of 60 μA with separator and source maintained at 280°.

Because the OV-17 chromatographic phase did not completely resolve the *bis*-TMSi-alk(en)ylcatechols from each other, and because individual mass channels accelerating voltage alternation) were not available on the GC–MS instrument short range magnetic scans were performed manually at regular timed intervals as the chromatographic peaks emerged from the GLC column. The mass range 350–420 was repetitively scanned at 6 sec intervals and about 10–15 scans per peak were obtained. The MS chart paper was folded in accordion fashion and the tops of the M^+ peaks corresponding to the pentadec(en)ylcatechol TMSi derivatives (*m/e* 458-464) and the heptadec(en)ylcatechol TMSi derivatives (*m/e* 486-492) were joined with a line (after correction for 13_C, 30_{Si}, and 29_{Si} contribution) to form a smooth elution profile. The areas of the peaks of the elution profile were determined by weighing tracings of the individual peaks. In separate GC–MS runs, the complete MS of each component was determined and the key ions were compared to those obtained with synthetic 3-*n*-pentadecylcatechol in order to verify that 3-*n*-alk(en)ylcatechols were responsible for the particular molecular ions.

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